DOCUMENT-IDENTIFIER: US 20020031820 A1

TITLE: Bisubstrate inhibitors of kinases

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Detail Description Paragraph - DETX (44):

[0055] An n-Alloc protecting group was added to the side chain amine of the commercially available diaminopropionic acid (DAP) residue by reacting DAP (500 mg: 1.5 mmol) with allyl chloroformate (163 uL: 1.5 mmol) in dimethylformamide (DMF) at room temperature for 24 hours. The protected residue was purified using silica gel size exclusion column chromatography (gradient elution MeOH: CH.sub.2Cl.sub.2). The eluting fractions were monitored using thin layer chromatography, mass spectrometry and NMR. The fractions containing DAP(n-alloc) were combined, dried under vacuum and used to synthesize the DAP(n-alloc) kemptide peptide. The DAP(n-alloc) kemptide peptide (FIG. 4) was prepared on a solid phase Wang resin (0.1 mmol scale) using Fmoc chemistry. The DAP(n-alloc) kemptide peptide was treated while on the solid phase with 70% CH.sub.2Cl.sub.2, 15% N-methyl morpholine, 15% acetic acid solution (5 mL) into which 450 mg tetrakis(triphenylphosphine) palladium (0) was added. This deprotection reaction was done under argon at room temperature for two hours with gentle shaking. The reaction solution was removed using a Poly Prep.RTM. chromatography filter column (BioRad) and the resin was washed with CH.sub.2Cl.sub.2 (50 mL) before rinsing with a palladium chelating solution consisting of 20 mL DMF, 225 mg diethyldithiocarbamic acid:3H.sub.2O and 250 uL
triethylamine (TEA). The resin was washed with 5%(v/v) TEA in DMF (50 mL) then methanol (50 mL) and dried. Bromoacetic acid (342 mg) was dissolved in a solution of DMF (5 mL) and diisopropylcarbodiimide (0.4 mL), added to the dried resin and allowed to react with the peptide for 5 hours at room temperature with gentle shaking. The resin was filtered and washed as described above, dried and then treated with a cleavage/deprotection solution (5 mL trifluoroacetic acid, 1.5 mL CH.sub.2Cl.sub.2, 250 (L ddH.sub.2O, 100 (L thioanisole) for 1 hr at room temperature. The released peptide was filtered from the resin, vacuum concentrated and then precipitated using cold ether (25 mL). The precipitate was dried under vacuum, dissolved in 7 mL of water and purified using reverse-phase preparative HPLC (gradient elution: H.sub.2O:CH.sub.3CN:0.05% trifluoroacetic acid (v/v), UV analysis at 214 nm) to give 7.9 mg of bromoacetylated peptide. The bromoacetylated product was dissolved in H.sub.2O (2 mL) and reacted with ATP.gamma.S (20 nmol) with

stirring for 24 hours at room temperature. The kemptide-ATP.gamma.S conjugate was purified from the reaction mixture using reverse phase preparative HPLC (gradient elution: H.sub.2O, CH.sub.3CN) and dried under vacuum to produce a final yield of 7 mg kemptide-ATP.gamma.S conjugate. The composition of the conjugate was confirmed using electrospray mass spectrometry and NMR data supported the proposed inhibitor structure. Concentration was determined using UV absorption at 260 nm.

US-PAT-NO: 5580968

DOCUMENT-IDENTIFIER: US 5580968 A

TITLE: DNA encoding phospholipase activating protein

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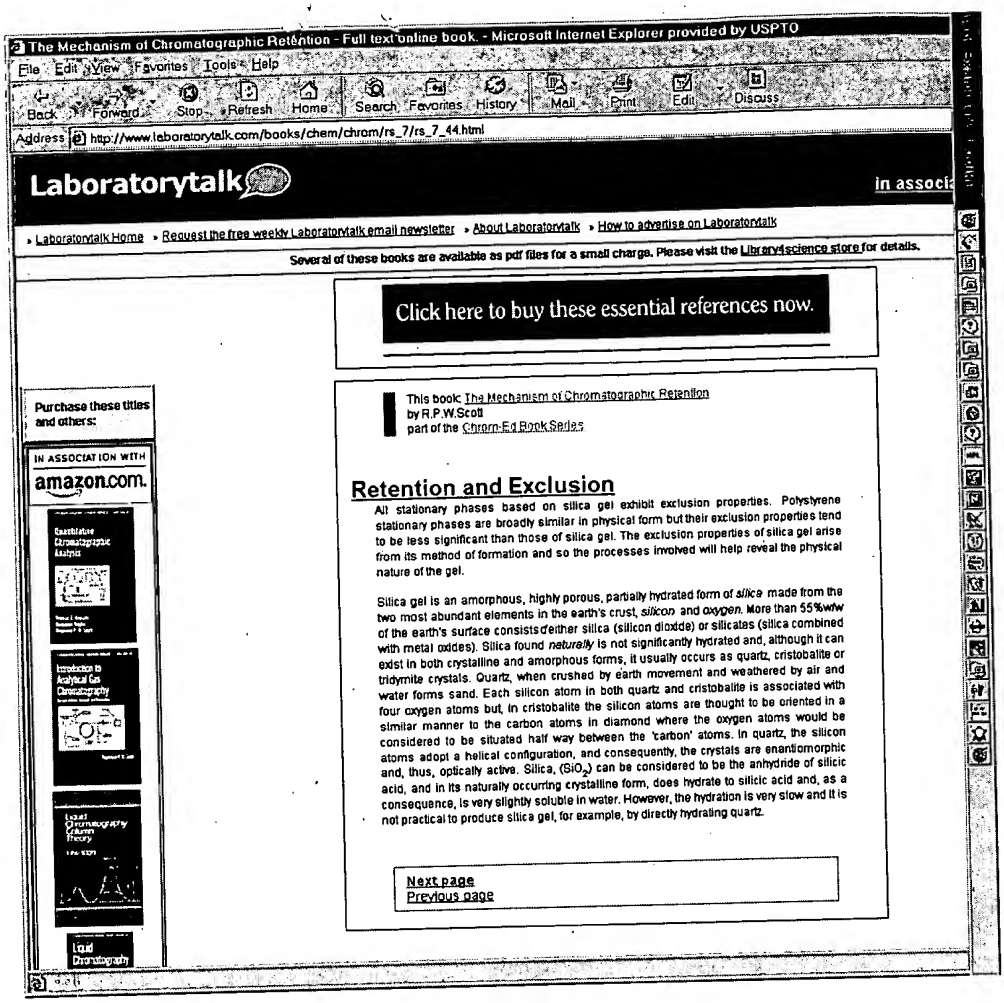
Detailed Description Text - DETX (18):

Purified human PLAP has an apparent molecular mass of about 28,000 as determined by SDS gel electrophoresis. PLAP may be purified from human synovial fluid or other human fluid or tissue containing the protein by affinity chromatography using immobilized anti-mellitin antibodies or antibodies specific for PLAP, followed by high pressure liquid chromatography (HPLC) using a size exclusion gel, preferably a silica gel, or any other conventional method, or combination of methods for isolating proteins that results in substantially purified PLAP. Preferred methods for isolating PLAP from human synovial fluid are described in detail in the Examples. PLAP is preferably isolated from synovial joint fluid of persons having rheumatoid arthritis, as the level of PLAP is higher in these persons. However, synovial fluid from persons not having rheumatoid arthritis is also suitable, although larger quantities of synovial fluid may be necessary due to the comparatively lower amount of PLAP in the synovial fluid of persons not having rheumatoid arthritis. Methods for affinity chromatography, high pressure liquid chromatography and other methods useful for isolating proteins may be found in the scientific literature and standard texts in the field such as Scopes, R. K., Protein Purification, Principles and Practice, second edition, Springer-Verlag, New York, N.Y. 1987.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Retention and Exclusion
All stationary phases based on silica gel exhibit exclusion properties. Polystyrene stationary phases are broadly similar in physical form but their exclusion properties tend to be less significant than those of silica gel. The exclusion properties of silica gel arise from its method of formation and so the processes involved will help reveal the physical nature of the gel.
Silica gel is an amorphous, highly porous, partially hydrated form of *silica* made from the two most abundant elements in the earth's crust, *silicon* and *oxygen*. More than 55%w/w of the earth's surface consists either silica (silicon dioxide) or silicates (silica combined with metal oxides). Silica found *naturally* is not significantly hydrated and, although it can exist in both crystalline and amorphous forms, it usually occurs as quartz, cristobalite or tridymite crystals. Quartz, when crushed by earth movement and weathered by air and water forms sand. Each silicon atom in both quartz and cristobalite is associated with four oxygen atoms but, in cristobalite the silicon atoms are thought to be oriented in a similar manner to the carbon atoms in diamond where the oxygen atoms would be considered to be situated half way between the 'carbon' atoms. In quartz, the silicon atoms adopt a helical configuration, and consequently, the crystals are enantiomorphic and, thus, optically active. Silica, (SiO_2) can be considered to be the anhydride of silicic acid, and in its naturally occurring crystalline form, does hydrate to silicic acid and, as a consequence, is very slightly soluble in water. However, the hydration is very slow and it is not practical to produce silica gel, for example, by directly hydrating quartz.
Next page
Previous page

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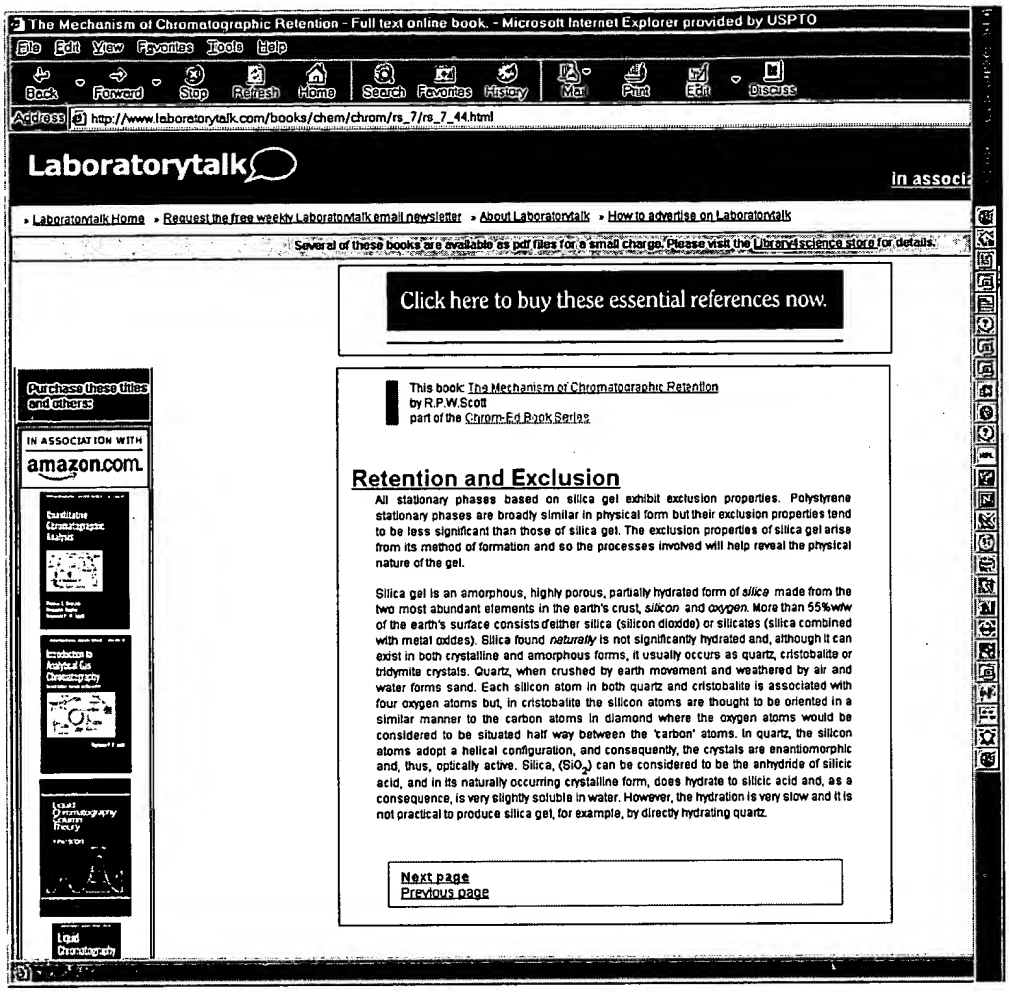
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Retention and Exclusion
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Next page
Previous page